

positive graft should be low after LT. The rate of carcinogenesis has increased at an annual rate of 0.11% after an SVR was maintained with anti-viral therapy [10]. Using a graft with a positive hepatitis C virus (HCV) antibody although HCV-RNA was not detected in the blood remains controversial in Western countries [11-13]. To the best of our knowledge, there is no actual data that shows the outcome of liver transplantation with a graft from patients who acquire an SVR after successful anti-viral therapy. Here, we report the case of a living donor who had an SVR before LDLT. The case is described and discussed in detail.

Case presentation

A 46-year-old Japanese woman donated the right lobe of her liver to her 36-year-old brother, who had decompensated cirrhosis of unknown origin. She experienced mild liver dysfunction (117IU/L in alanine aminotransferase, normal range of 5 to 30IU/L) eight years before the donation. Her condition was diagnosed as chronic active HCV infection (serotype 2) on the basis of a liver biopsy and viral study that showed that her level of HCV-RNA was 13 kcopy/mL by real-time polymerase chain reaction analysis. The histological diagnosis showed chronic hepatitis A1/F1 (Figure 1). She received anti-viral therapy with intra-muscular interferon α -2b three times weekly for 24 weeks. Her serum HCV assay results were negative after two weeks of effective anti-viral therapy.

She was doing well and no HCV-RNA had been detected. She maintained an SVR without any complications until she was evaluated as a living donor. The donor evaluation revealed anti-HCV antibody, but her liver function test results were normal and HCV-RNA was negative by polymerase chain reaction analysis. She underwent an ultrasound-guided needle biopsy of her liver, and the pathological findings were normal and there were no findings of active hepatitis (Figure 2). She was approved as a living donor after a thorough evaluation by the ethics committee of the Nagasaki University Graduate School of Biomedical Sciences. She was discharged nine days after the liver donation. Her liver function recovered immediately. A computed tomography scan one year later showed that she had sufficient liver regeneration. Her brother was also doing well after the LT and had no HCV infection 40 months after surgery and no *de novo* malignancy.

Discussion

Selecting a living donor in this case might be controversial, although a marginal donor can also sometimes be a candidate. The risks in this case included HCV transmission to the recipient, HCV reactivation in the recipient after LDLT, and donor risk during surgery. A number of studies have reported that the results with recipients of an HCV-infected graft were comparable to those of recipients of an HCV-negative graft [11-13]. The Scientific Registry of the United Network for Organ

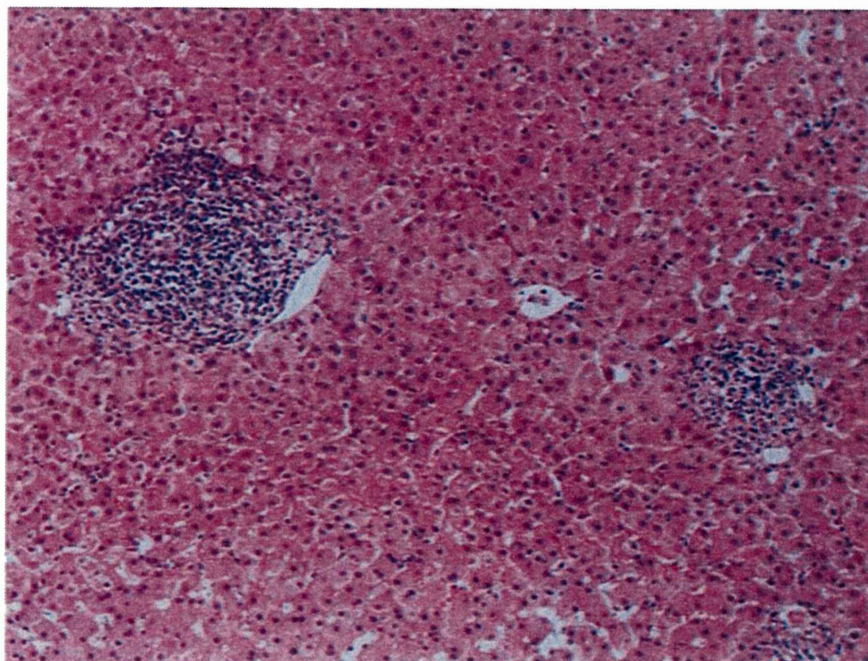


Figure 1 A liver biopsy showed chronic active hepatitis A1/F1 before interferon therapy.

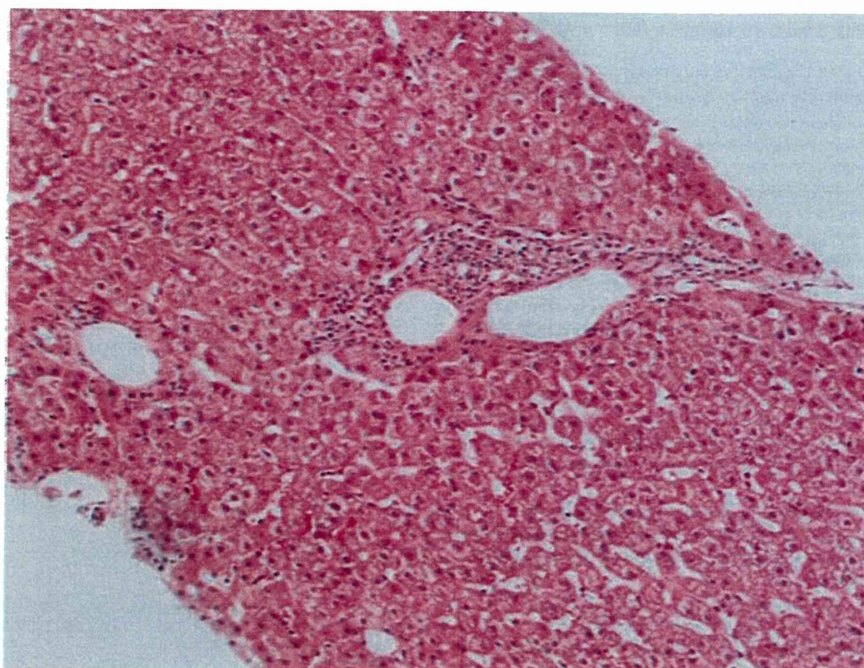


Figure 2 A liver biopsy shows normal liver tissue without hepatitis before the liver donation.

Sharing reported that the survival rate of 96 patients was significantly higher in the recipients of HCV-positive grafts than in recipients of HCV-negative grafts [13]. These results demonstrated that the use of an HCV-positive graft may also be acceptable in cadaveric LT. In contrast, an HCV-infected graft was not acceptable in LDLT. Patients who have HCV and who acquire an SVR after interferon therapy can be considered living donor candidates.

In this case, it was difficult to determine the indications for the donor selection before transplantation. Patients with serotype 2 HCV are more likely to achieve an SVR after interferon therapy than those with serotype 1. Our donor achieved an SVR of HCV. She and her brother were fully informed of the risk of peri-operative complications and the possibility that he would receive an HCV infection from the graft although she had obtained an SVR after anti-viral therapy. In theory, it is unlikely that a recipient would develop a viral infection from a graft that achieved an SVR.

Conclusions

We report a case of LDLT from a donor previously treated with interferon for HCV. A patient with SVR status after interferon therapy might be considered a candidate for LDLT only if there are no other possibilities of LT for the recipient. A careful follow-up of the donor after donation is needed. The recipient also must have a very close follow-up because it is difficult to predict what

might happen to the graft with post-transplant immunosuppression.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Abbreviations

DDLTL: deceased donor liver transplantation; HBV: hepatitis B virus; HCV: hepatitis C virus; LDLT: living donor liver transplantation; LT: liver transplantation; SVR: sustained viral response.

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Authors' contributions

MH and SE shared responsibility for the management of this patient and were involved in drafting the manuscript or revising it critically for important intellectual content. MT shared responsibility for the management of this patient. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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HEPATOLOGY

Ferritin/alanine aminotransferase ratio as a possible marker for predicting the prognosis of acute liver injuryEisuke Ozawa,^{*,†} Seigo Abiru,^{*} Shinya Nagaoka,^{*} Koji Yano,^{*} Atsumasa Komori,^{*} Kiyoshi Migita,^{*} Hiroshi Yatsushashi,^{*} Naota Taura,[†] Tatsuki Ichikawa,[†] Hiromi Ishibashi^{*} and Kazuhiko Nakao[†]^{*}Clinical Research Center, National Hospital Organization Nagasaki Medical Center, Omura, and [†]Department Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan**Key words**

acute liver failure, ferritin, heme oxygenase-1 (HO-1), prognostic predictor.

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Abstract**Background and Aims:** Serum levels of ferritin and heme oxygenase (HO)-1 are both markers of macrophage activation. We evaluated simple markers for predicting the prognosis of severe acute liver injury in which macrophage activation plays an important role.**Methods:** Subjects comprised 114 patients with acute liver injury, admitted to the liver unit of Nagasaki Medical Center between January 2001 and September 2010. Subjects included 11 patients with fulminant hepatic failure (FHF), 82 patients with ordinary acute hepatitis (AH), and 21 patients with severe-form AH (AHS). We determined serum levels of ferritin, HO-1 and other biochemical makers, and analyzed relationships between clinical outcomes of patients and each of these parameters alone and in combination.**Results:** Median serum ferritin levels were significantly higher in FHF (25 900 ng/mL) and AHS (3060 ng/mL) than in AH (700 ng/mL; $P < 0.01$ each). Median HO-1 levels were also significantly higher in FHF (123 ng/mL) and AHS (51 ng/mL) than in AH (19 ng/mL; $P < 0.01$ each). Similarly, median ferritin/alanine aminotransferase (F/A) ratio was significantly higher in FHF (6.7) than in AHS (1.6, $P < 0.05$) or AH (0.5, $P < 0.01$). Among the 11 FHF patients, three recovered, seven died and one underwent liver transplantation. The ability of F/A ratio to distinguish non-survivors from survivors was analyzed using receiver operating characteristics curves. A cut-off level of 3.12 provided high sensitivity (87.5%) and specificity (81.2%).**Conclusion:** These results suggest that F/A ratio offer a quick and simple marker for predicting the prognosis of acute liver injury.**Introduction**

Fulminant hepatic failure (FHF) is associated with high mortality rates, despite recent advances in medical management. In contrast, outcomes for acute hepatitis (AH), and even severe-form AH (AHS), are not fatal. The pathogenesis of FHF has not been elucidated in detail, but antigen-specific cytotoxic T lymphocytes, polyclonal cytokines, immune modulators, and products of oxidative stress have been shown to induce damage and destruction of hepatocytes in these patients.¹

Activated macrophages have been suggested to play important roles in the pathogenesis of FHF, as reflected by the activation of both pro- and anti-inflammatory cascades in the innate immune system.² Corticosteroids have been used to suppress macrophage activation in the treatment of severe acute hepatic failure.³ Recent reports have found that serum concentrations of interleukin (IL)-10 and soluble-form CD163 (sCD163), a macrophage-activating factor, are both highly elevated in FHF.^{4,5} Macrophages and their expression of Fas ligands may also play important roles in the pathogenesis of FHF.⁶

Ferritin, on the other hand, is a ubiquitous and highly conserved iron-binding protein. The serum ferritin level is an indicator of iron stores, and is also used as a marker of macrophage activation. Very high serum ferritin levels are observed in macrophage activation syndromes (MASs), such as hemophagocytic syndrome (HPS) and adult-onset Still's disease (AOSD), although the mechanisms underlying this increase are unclear.^{7,8} Iron metabolism is known to be regulated by iron-responsive proteins (IRPs) affecting ferritin at the mRNA level. Inflammation dramatically affects iron metabolism and a variety of inflammatory mediators act via IRPs.⁷

Heme oxygenase (HO) is an enzyme that catalyzes the conversion of heme into carbon monoxide (CO), Fe²⁺ and biliverdin. HO-1, an inducible form of HO, is a 32-kD heat-shock protein that is expressed in response to various noxious stimuli, including heavy metals, hyperoxia, hypoxia, endotoxin, hydrogen peroxide, and inflammatory cytokines.⁸⁻¹² Recent studies have identified serum HO-1 as a novel marker for diagnosing macrophage activation state under conditions such as sepsis, HPS, and AOSD.^{13,14}

Hepatocyte growth factor (HGF) was first discovered as a potent mitogen for adult hepatocytes.¹⁵ The significance of evaluating serum HGF levels in liver diseases has been addressed in patients with FHF, who show markedly increased levels of serum HGF.^{16,17} Measurement of serum HGF levels, which is commonly performed in Japan, may thus be useful for predicting fulminant progression and prognosis of acute liver disease.¹⁸

The difficult decision of whether to perform liver transplantation for patients with FHF should be made in the early stage of the disease.¹⁹ The King's College criteria have been widely applied,²⁰ but offers unacceptably low predictive accuracy.²¹ The aim of the present study was to identify a simple marker such as ferritin, F/A ratio and HO-1 for predicting the prognosis of acute liver injury in relation to serum HGF and to clarify the involvement of macrophage activation in FHF.

Methods

Inclusion criteria

Subjects in the present study were patients with AH, AHS or FHF. AH was diagnosed as an acute increase in levels of serum alanine aminotransferase (ALT) to > 10 times the upper limit of normal, with or without an increase in total bilirubin level. AHS was defined as AH without hepatic encephalopathy in addition to prothrombin activity < 40% of normal control or international normalized ratio (INR) > 2.0. FHF is defined most widely as a potentially reversible condition resulting from severe liver injury, with onset of encephalopathy occurring within 8 weeks after the symptom onset and in the absence of pre-existing liver disease.²² In this study, FHF was defined in accordance with Japanese criteria: development of hepatic encephalopathy of grade II or above within 8 weeks after the symptom onset in addition to prothrombin activity < 40% of normal control or > INR 2.0.

Patient management

Plasma exchange and blood filtration were performed for all FHF patients using a membrane plasma separator in addition to intensive total care management, including hemodynamic monitoring, mannitol therapy for cerebral edema, infusion of an H₂ antagonist, and nutritional support. AHS patients received similar intensive total care management, with the exception of extracorporeal circulation.

Patients

Subjects comprised 81 patients who were admitted with acute liver injury between January 2001 and September 2010 to the Liver Unit at Nagasaki Medical Center, Omura, Nagasaki, Japan. Of these, 11 patients were diagnosed with FHF, 21 patients with AHS, and 84 patients with AH. Of the 11 FH patients, three recovered, seven died and one underwent liver transplantation.

This study was performed after obtaining written informed consent from each patient or the appropriate guardian in accordance with the Ethics Guidelines for Clinical Study issued by the Ministry of Health, Labor and Welfare in Japan.

Biochemical assays

Blood samples were obtained on admission for analysis of biochemical data, including ALT, ferritin and HGF, and serum samples were stored at -80°C until use. HO-1 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (Human HO-1 ELISA kit; Stressgen, Ann Arbor, MI, USA). In brief, mouse monoclonal rabbit anti-HO-1 was coated onto microtiter wells. Each sample of 100 µL (diluted 1:20 in sample diluent) was added and incubated for 30 min. After washing, 100 µL of anti-human HO-1 antibody (diluted 1:500) was added and incubated for 1 h. After further washing, 100 µL of anti-rabbit immunoglobulin (Ig) G conjugated with horseradish peroxidase (diluted 1:4000) was added and incubated for 30 min. Wells were washed, and 100 µL of stabilized tetramethylbenzene substrate solution was added. After 15 min, 100 µL of acid stop solution was added, and plates were read at 450 nm. Control samples and standards of purified HO-1 were co-analyzed in each run. Inter-assay coefficient of variation (CV) was < 10%.

Serum concentrations of HGF were determined using commercially available ELISA kits (Otsuka, Tokyo, Japan), with absorbance read at 490 nm on an ELISA plate reader (Molecular Devices E-max, Concord, ON, Canada).

Statistical analysis

Non-parametric tests were used for comparisons between groups (Mann-Whitney test for unpaired data, Kruskal-Wallis test for comparisons among three groups) and for correlation analysis (Spearman ρ). The 95% confidence intervals for the area under the curve (AUC) in receiver operating characteristics (ROC) curves were calculated non-parametrically. All tests were two-sided, and values of $P < 0.05$ were considered significant. All statistical analyses were performed using Stat-flex version 5.0 software (Artech, Osaka, Japan).

Results

Clinical features

Clinical features of subjects are shown in Table 1. No significant differences in age and sex were seen among FHF, AHS and AH groups. Median serum ALT levels did not differ significantly among patients with FHF (5168 IU/L; range, 349–9670 IU/L) AHS (2802 IU/L; range, 400–13 200 IU/L) and AH (1523 IU/L; range, 327–5050 IU/L) ($P < 0.01$ each). The total serum bilirubin level was significantly higher in FHF (11.9 mg/dL; range, 2.5–32.2 mg/dL) than in AHS (7.0 mg/dL; range, 1.2–32.9 mg/dL) or AH (3.9 mg/dL; range, 0.3–34.0 mg/dL) ($P < 0.01$ each). Prothrombin activity was lowest in FHF (18.2%; range, 7.3–47.7%). FHF was caused by viral hepatitis in three patients (hepatitis A, $n = 1$; hepatitis B, $n = 1$; Epstein-Barr virus, $n = 1$), severe alcoholic hepatitis in two patients, hematological malignancy in three patients, acute heart failure in one patient, drug-induced hepatitis in one patient and indeterminate cause in one patient. AHS was caused by viral hepatitis in 10 patients (hepatitis A, $n = 6$; hepatitis B, $n = 4$), severe alcoholic hepatitis in one patient, drug-induced hepatitis in seven patients, and indeterminate cause in three patients. AH was caused by viral hepatitis in 41 patients (hepatitis

Table 1 Clinical characteristics of patients

	FHF (<i>n</i> = 11)	AHS (<i>n</i> = 21)	AH (<i>n</i> = 84)
Age (years)	64 (16–83)	48 (21–75)	45 (15–87)
Sex (male : female)	7:4	13:8	47:37
Total bilirubin (mg/dL)	11.9 (2.5–32.2)*	7.0 (1.2–32.9)	3.9 (0.3–34.0)
PT (%)	18.2 (7.3–47.7)**	37.0 (20.2–91.3)**	84.6 (48.9–120.8)
ALT (IU/L)	5168 (349–9670)	2 802 (400–13 200)	1523 (327–5050)
Plt (*10 ³ /μL)	143 (22–224)	149 (105–373)	185 (20–397)
Etiology			
Viral	3 (27.3%)	10 (47.6%)	41 (48.8%)
Hepatitis A	1	6	9
Hepatitis B	1	4	21
Other virus	1	0	11
Non-viral	8 (72.7%)	11 (52.4%)	43 (51.2%)
Drug	1	7	33
Other	7	4	10

Results are provided as median (range) or number (%).

P* < 0.05 versus AHS and *P* < 0.01 versus AH; *P* < 0.01 versus AH.

AH, acute hepatitis; AHS, severe-form acute hepatitis; ALT, alanine aminotransferase; FHF, fulminant hepatic failure; PT, prothrombin time.

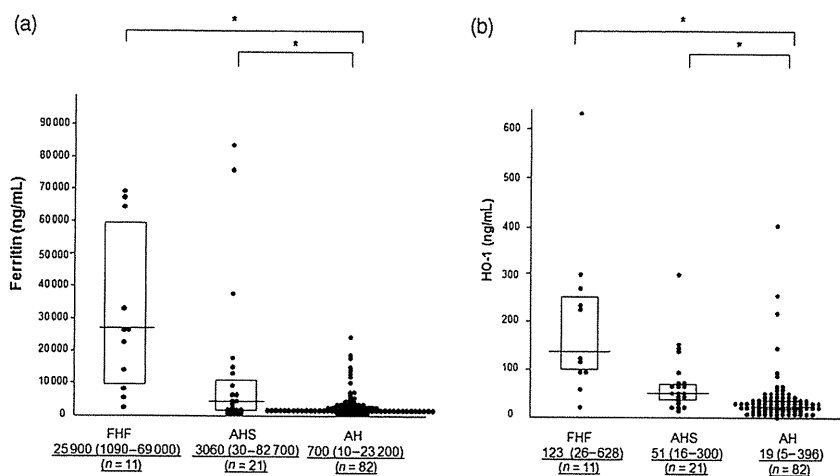


Figure 1 (a) Serum level of ferritin in subjects on admission. (b) Serum level of heme oxygenase (HO)-1 in subjects on admission. Box plots represent median and 25–75th percentiles. Upper and lower lines show minimum and maximum values, respectively. **P* < 0.01, as determined by the Kruskal–Wallis test.

A, *n* = 9; hepatitis B, *n* = 21; hepatitis C, *n* = 3; hepatitis E, *n* = 5; Epstein–Barr virus, *n* = 1; and cytomegalovirus, *n* = 1), drug-induced hepatitis in 33 patients, and indeterminate causes in eight patients.

Highly increased serum levels of ferritin and HO-1 in FHF and AHS

Median levels of serum ferritin on admission were significantly higher in FHF (25 900 ng/mL; range, 1090–69 000 ng/mL) and AHS (3060 ng/mL; range, 30–82 700 ng/mL) than in AH (700 ng/mL; range, 10–23 200 ng/mL; *P* < 0.01). However, no significant difference was identified between FHF and AHS (Fig. 1a). Similarly, median serum HO-1 levels on admission were significantly higher in FHF (123 ng/mL; range, 26–628 ng/mL) and AHS (51 ng/mL; range, 16–300 ng/mL) than in AH (19 ng/mL; range, 5–396 ng/mL; *P* < 0.01), and no significant

difference was apparent between FHF and AHS (Fig. 1b). HO-1 levels correlated significantly with ferritin levels (*r* = 0.57, *P* < 0.01; data not shown).

Highly increased ferritin/ALT (F/A) ratio in FHF and AH

Since ferritin is released into the circulation not only from activated macrophages but also from damaged hepatocytes,^{23,24} distinguishing the origin of ferritin in serum is difficult in patients with acute liver injury. We therefore evaluated the F/A ratio, as a reflection of the fraction of ferritin released from activated macrophages rather than that released from hepatocytes. Median F/A ratio was significantly higher in FHF (6.7; range, 1.7–13.2) than in AHS (1.6; range, 0.0–8.6; *P* < 0.05) or AH (0.5; range, 0.0–7.0; *P* ≤ 0.01 each) (Fig. 2a).

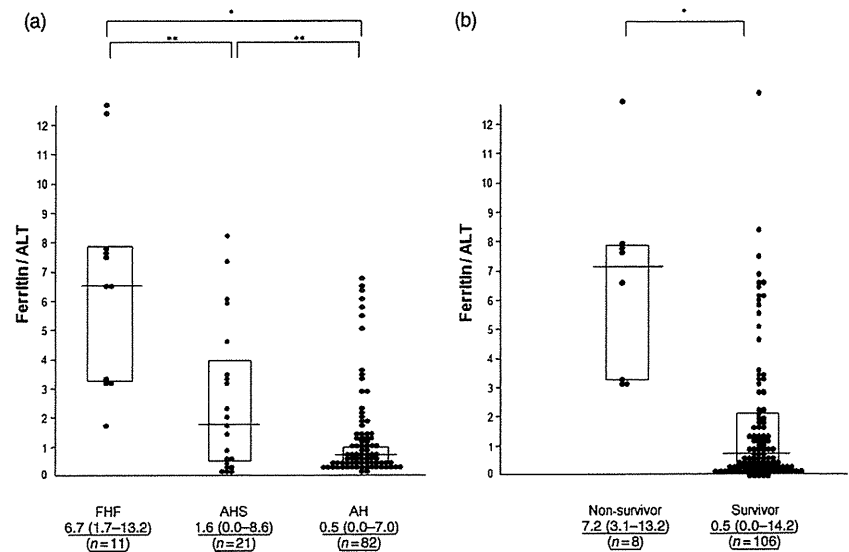


Figure 2 (a) Ferritin/alanine aminotransferase (ALT) ratio in subjects on admission. (b) Ferritin/ALT ratio in non-survivors and survivors on admission. * $P < 0.01$, ** $P < 0.05$, as determined by the non-paired Mann–Whitney U -test.

Table 2 Comparisons of various parameters between survivors and non-survivors as assessed by univariate analysis

	Survivors ($n = 108$)	Non-survivors ($n = 8$)	P -value
Age (years)	46.0 (15–87)	64.0 (16–83)	n.s.
Sex (male : female)	61:47	6:2	n.s.
Type (AH : AHS : FHF)	84:21:3	0:0:8	< 0.01
Total bilirubin (mg/dL)	4.20 (3.00–32.2)	15.4 (0.30–34.0)	< 0.01
PT (%)	76.9 (15.2–47.7)	33.1 (7.3–120.8)	< 0.01
ALT (IU/L)	1 787 (327–13 200)	4 227 (349–9 670)	n.s.
Plt ($\times 10^3/\mu\text{L}$)	137 (20–397)	46.8 (22–191)	< 0.01
Viral/non-viral (n)	52/56	2/6	n.s.
Ferritin (ng/mL)	1 025 (14–82 700)	23 800 (1 090–66 900)	< 0.01
HO-1 (ng/mL)	34.0 (5.0–1 048.5)	106.0 (26.0–628.0)	< 0.01
F/A ratio	0.7 (0.01–13.2)	7.2 (3.12–12.95)	< 0.01
HGF (ng/mL)	0.5 (0.22–8.0)	5.0 (1.60–9.94)	< 0.01

Results are provided as median (range) or number.

ALT, alanine aminotransferase; FA ratio, ferritin/alanine aminotransferase ratio; HGF, hepatocyte growth factor; HO-1, heme oxygenase-1; n.s., not significant; Plt, platelet count; PT, prothrombin time.

Predicting survival by ferritin, HO-1, and F/A ratio

When we compared non-survivors ($n = 8$) and survivors ($n = 106$), median F/A ratio was significantly higher in non-survivors (7.2; range, 3.1–13.2) than in survivors (0.5; range, 0.0–14.2; $P < 0.01$). F/A ratio was > 3.0 in all non-survivors and liver-transplanted patients (Fig. 2b).

Comparisons of various parameters between survivors and non-survivors are shown in Table 2. Median serum ferritin level on admission was significantly higher in non-survivors (23 800 ng/mL; range, 1090–66 900 ng/mL) than in survivors (1025 ng/mL; range, 14–82 700 ng/mL; $P < 0.01$). Median serum HO-1 level was also significantly higher in non-survivors (106.0 ng/mL; range, 26.0–628.0 ng/mL) than in survivors (34.0 ng/mL; range, 5.0–1048.5 ng/mL; $P < 0.01$). No significant differences in age,

sex, etiology or ALT level were seen among these three groups. Serum total bilirubin levels were significantly higher in non-survivors than in survivors, whereas prothrombin activities were significantly lower in non-survivors than in survivors. Median serum HGF levels were significantly higher in non-survivors (5.0 ng/mL; range, 1.60–9.94 ng/mL) than in survivors (0.5 ng/mL; range, 0.22–8.0 ng/mL; $P < 0.01$).

The ability to distinguish non-survivors from survivors was analyzed by generating ROC curves for sensitivity and specificity using different cut-off levels (Fig. 3, Table 3). For F/A ratio, a cut-off of 3.12 generated 87.5% sensitivity and 81.2% specificity. The odds ratio for the F/A ratio was 30.1. For serum HGF levels, a cut-off of 1.60 ng/mL generated 87.5% sensitivity and 94.1% specificity. The odds ratio for the serum HGF level was 110.0. The odds ratios for the F/A ratio and serum HGF level were higher than or approximately equivalent to those for bilirubin, prothrombin

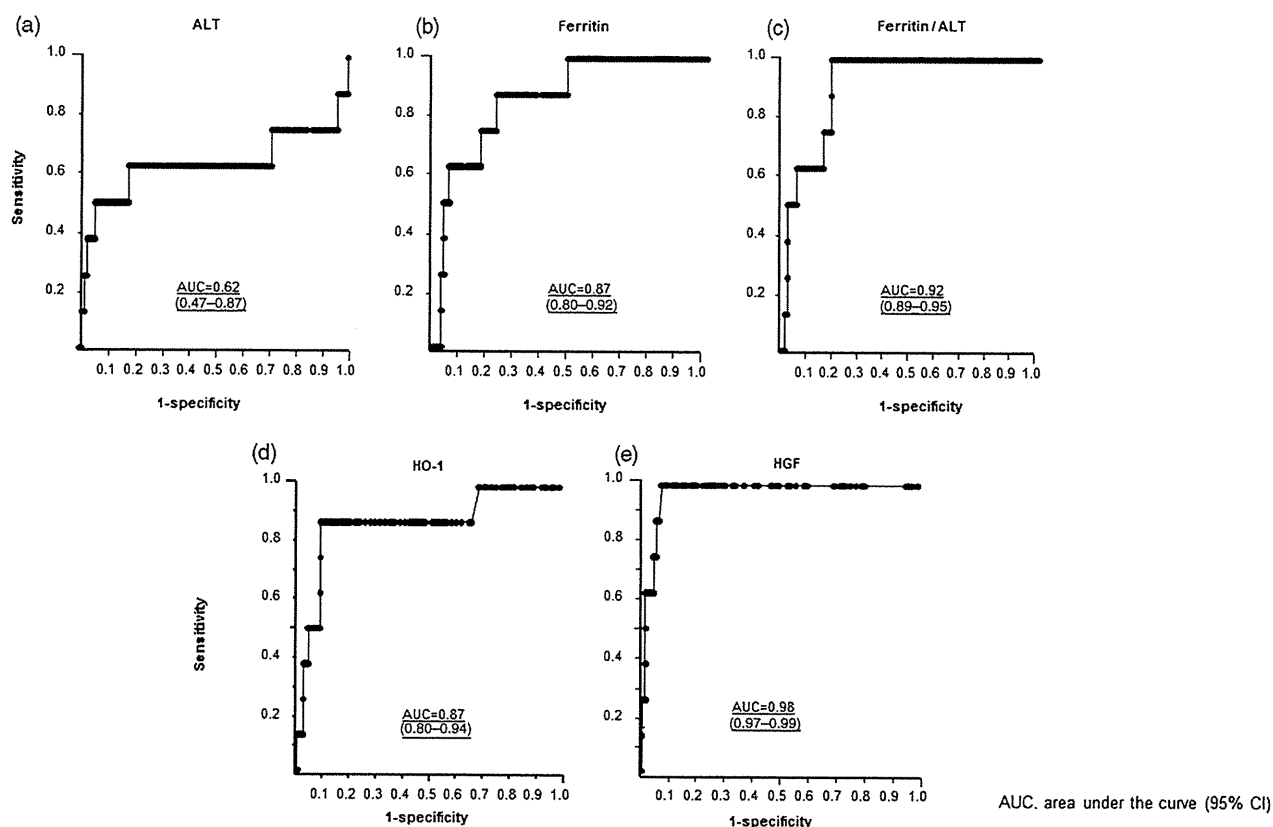


Figure 3 Receiver operating characteristics (ROC) curve for predicting fatal outcome in patients with acute liver failure. (a) Alanine aminotransferase (ALT) level on admission. (b) Serum ferritin level on admission. (c) Ferritin/ALT ratio on admission. (d) Serum HO-1 level on admission. (e) Serum hepatocyte growth factor (HGF) level on admission. AUC, area under the curve (95% confidence interval).

Table 3 Ability of ferritin/ALT ratio to distinguish non-survivors from survivors as described by ROC curves

	Cut off	Sensitivity	Specificity	PPV	NPV	Likelihood ratio	Odds ratio
ALT (IU/L)	2888	62.5%	74.4%	15.1%	96.2%	2.36	4.64
T. bil (mg/dL)	11.8	62.5%	80.6%	20.0%	96.5%	3.21	6.91
Cr (mg/dL)	0.9	37.5%	87.7%	17.6%	94.7%	2.81	3.90
PT (%)	33.8	57.1%	95.2%	28.5%	96.9%	6.0	12.6
Plt ($\times 10^3/\mu\text{L}$)	122	60.0%	85.9%	16.6%	97.8%	4.24	9.1
Ferritin (ng/mL)	4040	87.5%	77.4%	22.5%	98.7%	3.86	23.9
F/A ratio	3.12	87.5%	81.2%	25.9%	98.9%	4.63	30.1
HO-1 (ng/mL)	97.0	87.5%	92.5%	43.7%	99.0%	10.3	75.4
HGF (ng/mL)	1.60	87.5%	94.1%	53.3%	98.9%	14.7	110.0

ALT, alanine aminotransferase; F/A ratio, ferritin/alanine aminotransferase ratio; HGF, hepatocyte growth factor; HO-1, heme oxygenase 1; NPV, negative predictive value; PPV, positive predictive value.

time, platelet count and creatinine. AUCs for both F/A ratio and serum HGF levels were > 0.9 .

Predicting prognosis using a combination of F/A ratio and serum HGF level

As both F/A ratio and serum HGF level were correlated with clinical outcomes for patients with FHF, we analyzed F/A ratio

together with the serum HGF level. In the liver-transplanted patient and patients who died (black dots in Fig. 4), HGF level was ≥ 1.0 ng/mL and F/A ratio was ≥ 3.0 . For predicting mortality, the combined parameter of F/A ratio ≥ 3.0 and HGF ≥ 1 ng/mL showed superior specificity and likelihood ratio (sensitivity, 100%; specificity, 94.3%; likelihood ratio, 17.7) than the single parameter of HGF ≥ 1.0 ng/mL (sensitivity, 100%; specificity, 81.1%; likelihood ratio, 5.3) (Table 4).

Discussion

The decision whether to perform liver transplantation for FHF patients is critical, but also often difficult.¹⁹ Marker molecules in more robust prognostic models may thus have important clinical value.

Several reports have noted that macrophage-related factors may play a dominant role in determining the severity of disease in patients with FHF.^{4-6,23} Expression of osteopontin is high in Kupffer cells and hepatic macrophages in rat liver after carbon tetrachloride intoxication.²⁵ Expression of CD163 in liver tissue is higher in patients with acute viral hepatitis than in those with chronic viral hepatitis.^{26,27} Serum levels of IL-10 and tumor necrosis factor- α are high in patients with FHF and correlate with risk of fatal outcomes.⁴ Serum levels of sCD163, a lineage-specific scavenger receptor regulated by IL-10 that is involved in several anti-inflammatory functions of the immune system, are significantly higher in patients with FHF compared to patients with AH²⁶ and again correlate with fatal outcomes.²⁸ *In vitro*, macrophages can take up different phenotypes dependent on the cytokine environment,²⁹ as reflected *in vivo* by pro- and anti-inflammatory activation states balancing the immune response. Taken together, the high levels of macrophage activation markers in FHF may represent an anti-inflammatory imbalance, particularly in patients with poor disease outcomes. The present results indicate that serum

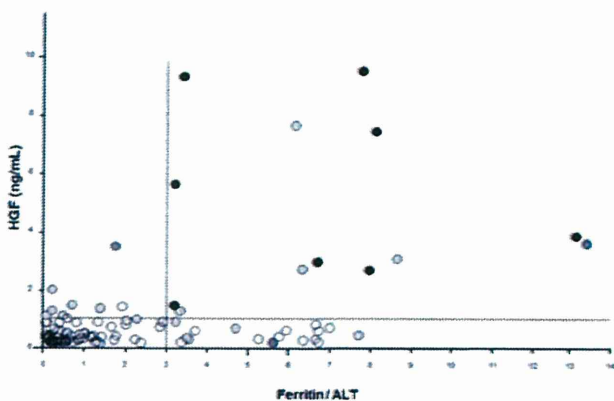


Figure 4 Ferritin/alanine aminotransferase (ALT) ratio with serum hepatocyte growth factor (HGF) level related to clinical outcomes. Horizontal dotted line indicates HGF at 1.60 ng/mL, as determined on the basis of receiver operating characteristics (ROC) curve. Vertical dotted line indicates an arbitrary cutoff value of 3.12 ng/mL for HO-1. ●, fulminant hepatic failure (FHF) non-survivor or transplantation; ●, FHF survivor; ○, severe-form acute hepatitis (AHS); ○, acute hepatitis (AH).

levels of ferritin and HO-1, both of which originate from activated macrophages, and are highly increased in acute liver injury.

As ferritin synthesis is stimulated by Fe²⁺, which is generated by HO-1-mediated heme degradation, hyperferritinemia might be caused by high HO-1 activity, irrespective of the underlying disease. Induction of HO-1 by extracellular heme has been shown to increase the free iron pool relevant for subsequent sequestration into ferritin.¹⁴

HO-1, an inducible heme-degrading enzyme converting heme into CO, Fe²⁺, and biliverdin, is a 32-kD heat-shock protein. HO-1 is expressed by macrophages and endothelial cells in response to various noxious stresses, and plays an important role against oxidative injuries.¹⁰⁻¹² Recent studies have shown that in MAS such as HPS and AOSD, serum HO-1 levels correlate closely with serum ferritin levels. Serum HO-1 levels could thus prove useful in differential diagnosis of hyperferritinemia and perhaps also in monitoring disease activity.¹⁴ Although HO-1 expression was markedly increased at both transcriptional and protein levels in hepatocytes with a rat model of carbon tetrachloride-induced acute liver injury,³⁰ an increase in free heme concentration may upregulate HO-1 gene expression in patients with acute liver injury. In the present study, serum levels of ferritin and HO-1 were significantly higher in FHF and AHS than in AH, suggesting that activated macrophages may play a role in progression to FHF.

Serum ferritin levels are increased not only by release from hepatocytes as a result of liver damage, but also from activated macrophages.^{23,24,31} Distinguishing the origin of ferritin between activated macrophages and liver cell cytolysis may be difficult. A recent report demonstrated a high ferritin level with a low percentage of glycosylated ferritin in patients with MAS, such as HPS and AOSD.^{32,33} However, assaying glycosylated ferritin is not easy. We therefore determined the F/A ratio to reflect ferritin released from activated macrophages, revealing a significant difference in F/A ratio between FHF and AHS.

In clinical situations, assessing whether patients presenting with features of acute liver damage are likely to recover after an acute attack of hepatitis or will eventually develop FHF is very important. The present study found that serum levels of HO-1 and ferritin, both of which are macrophage-activation markers, were high in patients with FHF and AHS. These results suggest that activation of macrophages occurs in FHF and AHS, and that inflammatory cytokines can interact in the initiation and progression of liver cell damage. HO-1 and ferritin may be produced directly by activated macrophages in the liver of FHF patients, as macrophages play a dominant role in the pathogenesis of severe inflammation in FHF.

F/A ratio could reflect the amount of ferritin released from activated macrophages, and was significantly high in non-surviving patients with FHF in this study. In addition, the combi-

Table 4 Prognostic prediction by combining HGF and F/A ratio

	Non-survivors	Survivors	Total	Sensitivity	Specificity	Likelihood ratio
HGF \geq 1 ng/mL	8	20	28	100% (8/8)	81.1% (86/106)	5.3
HGF < 1 ng/mL	0	86	86			
HGF \geq 1 ng/mL and F/A ratio \geq 3	8	6	14	100% (8/8)	94.3% (100/106)	17.7
HGF < 1 ng/mL or F/A ratio < 3	0	100	100			

F/A ratio, ferritin/ALT ratio; HGF, hepatocyte growth factor.

nation of F/A ratio and serum HGF level offered a useful marker for predicting mortality from acute liver injury. However, these results may reflect both sides of the hepatocyte destruction and macrophage activation in FHF. Some limitations to the present study must be considered, such as the relatively small number of samples and the heterogeneous etiologies. Prospective studies are thus required to validate the predictive value of the markers identified.

In conclusion, our results suggest that activation of macrophages participates in the pathophysiology of acute liver injury, and that the combination of F/A ratio and serum HGF level offers a potent marker for predicting the severity and prognosis of acute liver injury.

Acknowledgments

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Letter to the Editor

Successful pegylated interferon alpha2a monotherapy for hepatitis C virus infection in a transplanted patient who relapsed after the preceding course

T. Ichikawa, N. Taura, H. Miyaaki, M. Matsuzaki, S. Eguchi, M. Takatsuki, T. Kanematsu, K. Nakao. Successful pegylated interferon alpha2a monotherapy for hepatitis C virus infection in a transplanted patient who relapsed after the preceding course. *Transpl Infect Dis* 2011; **13**: 438–440. All rights reserved

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Key words: HCV; IFN; liver transplantation

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To the Editor

The combination of pegylated interferon (peg-IFN) with ribavirin is the only treatment strategy at present for the treatment for hepatitis C virus (HCV) infection after liver transplantation (LT), but its effects are still incomplete. We successfully treated a patient with peg-IFN alpha2a (peg-IFN- α 2a) monotherapy who showed a relapse after treatment with peg-IFN combined with ribavirin.

A 55-year-old female with liver cirrhosis and hepatocellular carcinoma within the Milan criteria was referred to Nagasaki University Hospital in December 2005. On admission, she had no ascites or hepatic encephalopathy and was determined to have Child–Pugh score 7 and modified end-stage liver disease score 12.5. She had repeatedly ruptured esophageal varices. LT surgery was successfully performed.

After living-donor LT, tacrolimus and prednisone were administered at the standard doses used in the immunosuppressive protocol. Her laboratory data on post-operative day 15 were 58 U/L of aspartate aminotransferase (AST) and 110 U/L of alanine aminotransferase (ALT). A liver biopsy revealed fibrosis stage 1 and activity grade 2. The calcineurin inhibitor was switched to cyclosporine for IFN treatment with the combination of 80 μ g of peg-IFN- α 2b weekly with 600 mg of ribavirin daily. Her HCV was genotype 1a and 1,570,000 IU/mL at the start of IFN treatment. The IFN treatment was introduced at postoperative day 19. The first IFN treatment continued for 52 weeks, because the HCV-RNA titer had a 2-log decrease from the baseline at 12 weeks of IFN treatment and disappeared at 18 weeks, and HCV-RNA was positive at the end point of treatment.

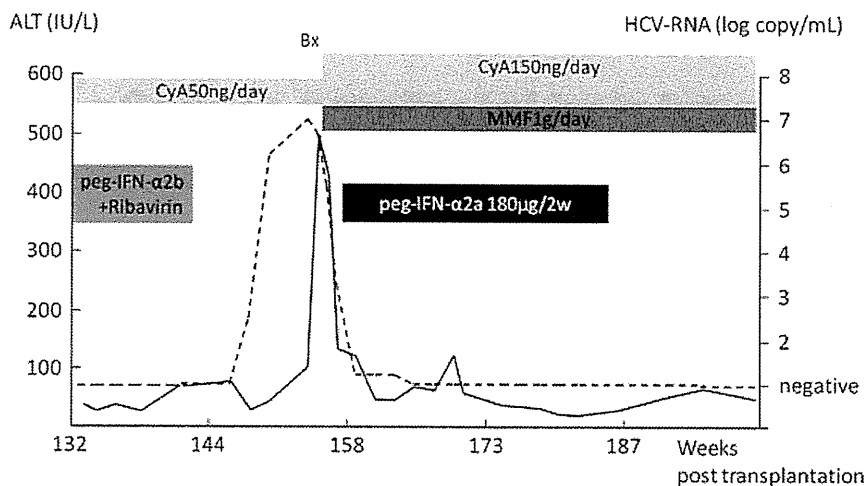


Fig. 1. Clinical course of rebound phenomenon after the second course of interferon (IFN) therapy. Solid lines are alanine aminotransferase (ALT) value (IU/L). Dotted lines are hepatitis C virus (HCV)-RNA value (log copy/mL). HCV-RNA is evaluated by the real-time polymerase chain reaction method. More than 1.2 log copy/mL of HCV-RNA is the sensitivity. Bx, liver biopsy; peg-IFN, pegylated interferon; w, weeks; CyA, cyclosporine (Neoral; Novartis Pharma, Tokyo, Japan); MMF, mycophenolate mofetil (Cellcept; Chugai, Tokyo, Japan).

After the first course, AST and ALT stabilized within the normal range and a liver biopsy revealed fibrosis stage 1 and activity score 1 at postoperative week 52. We waited for an improvement in the patient's side effects, and began the second treatment. The second course started at postoperative week 72, as her transplanted liver suffered from HCV-infected chronic hepatitis, according to the liver biopsy findings obtained at postoperative week 52. The second course was the combination of 90 µg of peg-IFN-α2a weekly with 200 mg of ribavirin daily for 9.5 g/dL of hemoglobin, because the early treatment of peg-IFN-α2b with ribavirin proved not to be effective (1).

An HCV-RNA qualitative assay, using the real-time polymerase chain reaction (PCR) method with a lower limit sensitivity of 15 IU/mL, was negative in serum at 20 weeks after IFN treatment. The HCV-RNA titer was positive in serum at 4 weeks after the end date of the second course, and reached a peak at 10 weeks after the second course. The ALT value indicated a relapse at 12 weeks. A liver biopsy revealed HCV-related chronic hepatitis. We introduced 180 µg of peg-IFN-α2a monotherapy once every 2 weeks, at 14 weeks after the second course.

HCV-RNA was negative by qualitative real-time PCR at 8 weeks after monotherapy. ALT was normalized at 4 weeks after monotherapy. The peg-IFN-α2a monotherapy was stopped at the treatment period of 6 months. HCV was negative at 6 months after the treatment end date, and she was determined to have a sustained viral response.

Some relapsing patients after IFN treatment show a flare up of HCV-RNA and ALT, the so-called 'rebound phenomenon' (2–6). ALT and HCV-RNA decrease again, after the rebound flare up. This is the best timing for induction of a second course of IFN (4, 5). The second course of IFN therapy has been reported to be IFN monotherapy for 6 months (4, 5). The second course of IFN monotherapy has a completion rate of 50% (4, 5). In our case, the third IFN monotherapy, but not the second IFN therapy, was induced at timing after rebound (Fig. 1).

The 'rebound phenomenon' is associated with immunological pressure against a rapid HCV increase (5, 7). A transplanted patient who shows relapse after the early treatment course must be treated quickly, because the HCV infection might be halted even by a single IFN treatment.

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Imaging of focal nodular hyperplastic-like nodules in alcoholic liver cirrhosis patients using gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging

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Abstract We report on two patients with a history of alcohol abuse who presented with multiple hepatic nodules. Dynamic computed tomography revealed multiple nodular lesions, which were enhanced at the early contrast phase and washed out at the portal phase. In the hepatobiliary phase using gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging (Gd-EOB-DTPA MRI), these tumors did not show any uptake, thus suggesting the presence of hepatocellular carcinoma. An ultrasound-guided biopsy revealed a slight increase in cell density, sinusoidal dilatation, and contained unpaired small arteries. According to immunohistochemical analyses, these arteries were positive for CD34 and alpha-smooth muscle actin. From these findings, the nodules were diagnosed to be focal nodular hyperplastic (FNH)-like nodules arising in alcoholic-cirrhotic livers. The differential diagnosis of FNH-like nodules arising in alcoholic liver cirrhosis and hepatocellular carcinoma is difficult with Gd-EOB-DTPA MRI, and therefore histological confirmation is necessary.

Keywords Focal nodular hyperplastic-like nodules ·
Alcoholic liver cirrhosis · Gd-EOB-DTPA MRI

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Introduction

Owing to the remarkable advances in various imaging techniques, an increased number of small nodular lesions are now being detected in the liver, and percutaneous fine-needle biopsy now makes it possible to perform a qualitative diagnosis of the detected lesions. However, Nakashima et al. [1] reported that focal nodular hyperplastic (FNH)-like nodules arising in patients with alcoholic liver cirrhosis could not be easily differentiated from well-differentiated hepatocellular carcinomas (HCCs), because the imaging results of FNH-like nodules and HCCs both reveal a high degree of hypervascularity. Recently, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) has been developed as a liver-specific magnetic resonance imaging (MRI) contrast agent [2–4]. In the hepatobiliary phase, hepatic lesions which lack normally-functioning hepatocytes are imaged, and then hepatocyte-selective uptake defects make it possible to differentiate HCCs and other benign hepatocellular nodules. However, few reports have so far examined the Gd-EOB-DTPA imaging findings of FNH-like nodules arising in patients with alcoholic liver cirrhosis. Here we outline two case reports and present the imaging findings using Gd-EOB-DTPA MRI for FNH-like nodules arising in patients with alcoholic liver cirrhosis, and show such nodules to have imaging characteristics similar to those of HCC.

Case reports

Case 1

A 47-year-old male patient was admitted to our hospital for further examination of multiple hepatic nodules. The

patient's alcohol consumption over 30 years was 120 g/day. At the time of admission, the patient's physical examination revealed a height of 160.8 cm, a weight of 37.2 kg, and a body temperature of 37.2°C. The relevant laboratory test results were as follows: platelet count 66,000/mm³; prothrombin time 69%; serum albumin 3.6 mg/dl; total bilirubin 1.6 mg/dl; serum aspartate transaminase (AST) 115 U/l; serum alanine transferase (ALT) 56 U/l; and the hepatitis B surface (HBs) antigen and hepatitis C virus (HCV) antibody were negative. The tumor markers were elevated as follows: α -fetoprotein (AFP) 10.2 ng/ml and protein induced by vitamin K absence or antagonist (PIVKA) II 285 mAU/m. The indocyanine green clearance revealed a high degree of retention (60%). Ultrasonography revealed small nodular lesions, detected as hypoechoic or isoechoic nodules. Dynamic computed tomography (CT) revealed multiple nodular lesions which demonstrated a slightly high degree of attenuation at the early contrast phase and washed out at the portal phase. Nodular lesions in the liver S2 showed peripheral low-attenuation areas, suggesting encapsulation (Fig. 1). By MRI, the peripheral part of the nodule in S2

showed a low intensity. The inside part of this nodule showed a high intensity in the T1 in-phase images, low intensity in the T1 out-phase images, and high intensity in the T2 weighted images, thus suggesting that these nodules were steatosis. In the hepatobiliary phase using Gd-EOB-DTPA, part of the tumors show a hypointensity area, suggesting HCC component (Fig. 2). Because the possibility of HCC was not ruled out, a percutaneous liver nodule biopsy was performed on the S2 nodule for a definitive diagnosis.

On microscopic examination, in comparison to the background liver, the nodules showed a slightly increased cell density, scar-like fibrosis, sinusoidal dilatation, and steatosis. The scar-like fibrosis contained unpaired small arteries showing sinusoidal capillarization (Fig. 3). Immunohistochemically, although part of tumor did not show OATP1B3 immunostaining (Fig. 4), several CD68-positive Kupffer cells were detected in the nodule (Fig. 3). From these findings, the nodules were diagnosed as FNH-like nodules arising in a patient with alcoholic liver cirrhosis.

Fig. 1 Computed tomography (CT) findings in case 1. The nodular lesion in liver S2 showed slightly low attenuation on the unenhanced CT (a), showed a slightly high degree of attenuation at the early contrast phase (b), was washed out at the portal phase (c), and was peripherally low-attenuated on dynamic CT (arrow)

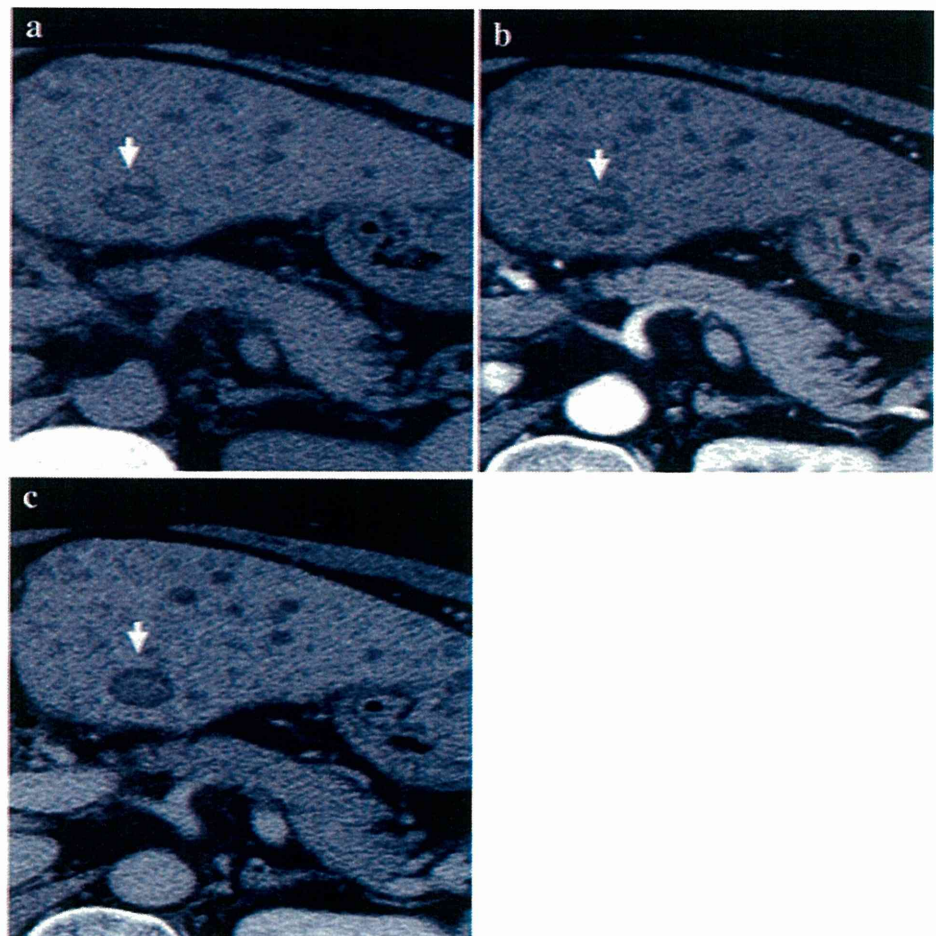
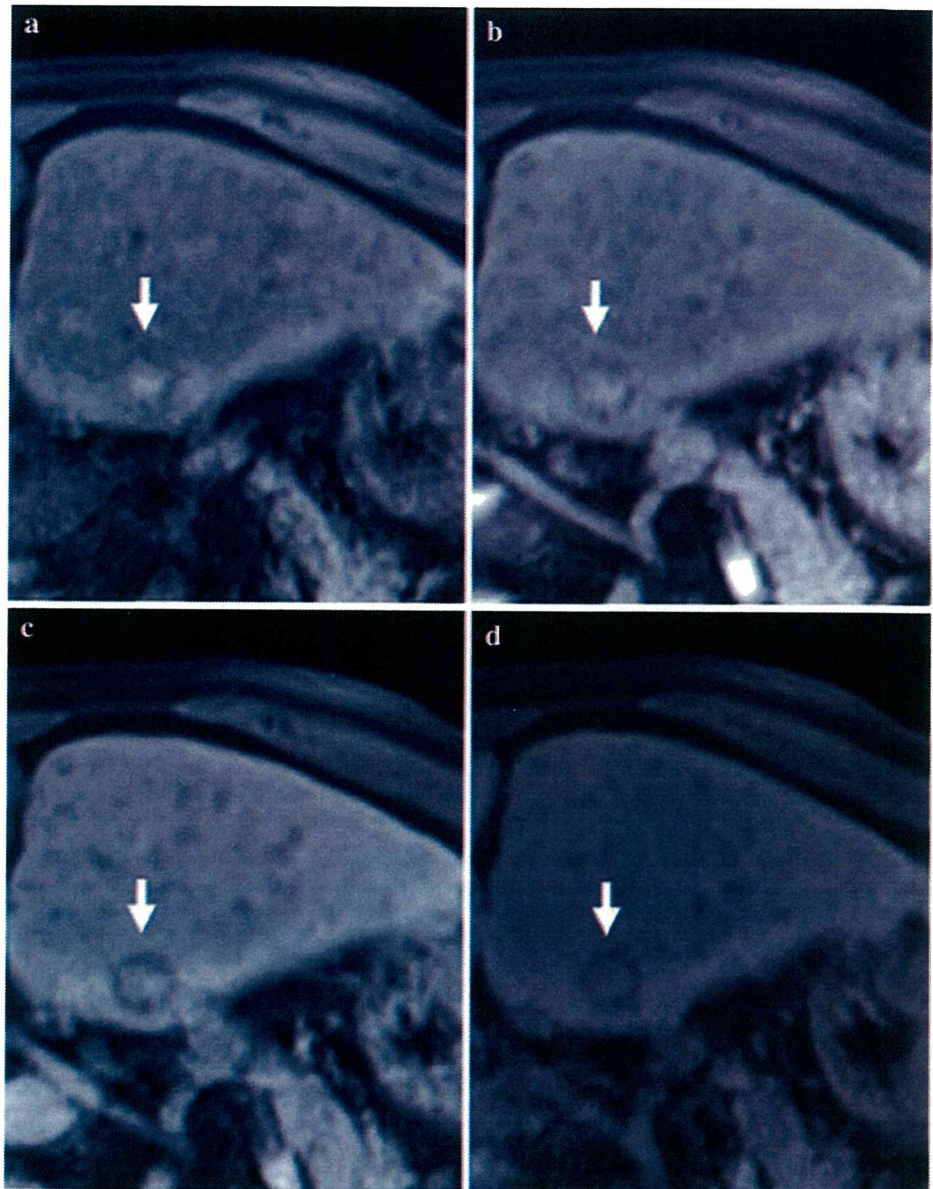


Fig. 2 Magnetic resonance imaging findings. The peripheral part of the nodule in S2 showed a low intensity. The inside part of this nodule showed a high intensity in the precontrast T1 weighted image (a), was slightly enhanced at the early contrast phase (b), and washed out at the portal phase (c). Part of the tumor showed defects in the hepatobiliary phase (d) (3D-gradient echo sequence (GRE) FS-T1W1, TR 4.30, TE 1.61, FA10)



Case 2

A 45-year-old female patient was admitted to our hospital for further examination of multiple hepatic nodules. The patient's alcohol consumption over 25 years was 180 g/day. At the time of admission, the physical examination revealed a height of 161.2 cm, a weight of 52.1 kg, and a body temperature of 36.3°C. The relevant laboratory test results were as follows: platelet count 96,000/mm³; prothrombin time 58%; serum albumin 3.3 mg/dl; total bilirubin 0.5 mg/dl; serum AST 49 U/l; serum ALT 36 U/l; and the HBs antigen and HCV antibody were negative. The tumor markers were elevated as follows: AFP 34.8 ng/ml and PIVKA II 11 mAU/m.

Contrast CT revealed multiple nodular lesions, which had a strong contrast enhancement at the early contrast phase and washed out at the portal phase. By MRI, the nodules showed a high intensity in the precontrast T1 weighted images and a low intensity in the T2 weighted images. In the hepatobiliary phase using Gd-EOB-DTPA, these nodules did not show any uptake (Fig. 5). From these findings, the possibility of a well-differentiated HCC was not ruled out, and a percutaneous liver nodule biopsy was performed for a definitive diagnosis.

On microscopic examination, compared with the background liver, the nodules showed a moderate increase in cell density and a scar-like fibrosis (Fig. 6) which contained artery-like vessels. Immunohistochemically,

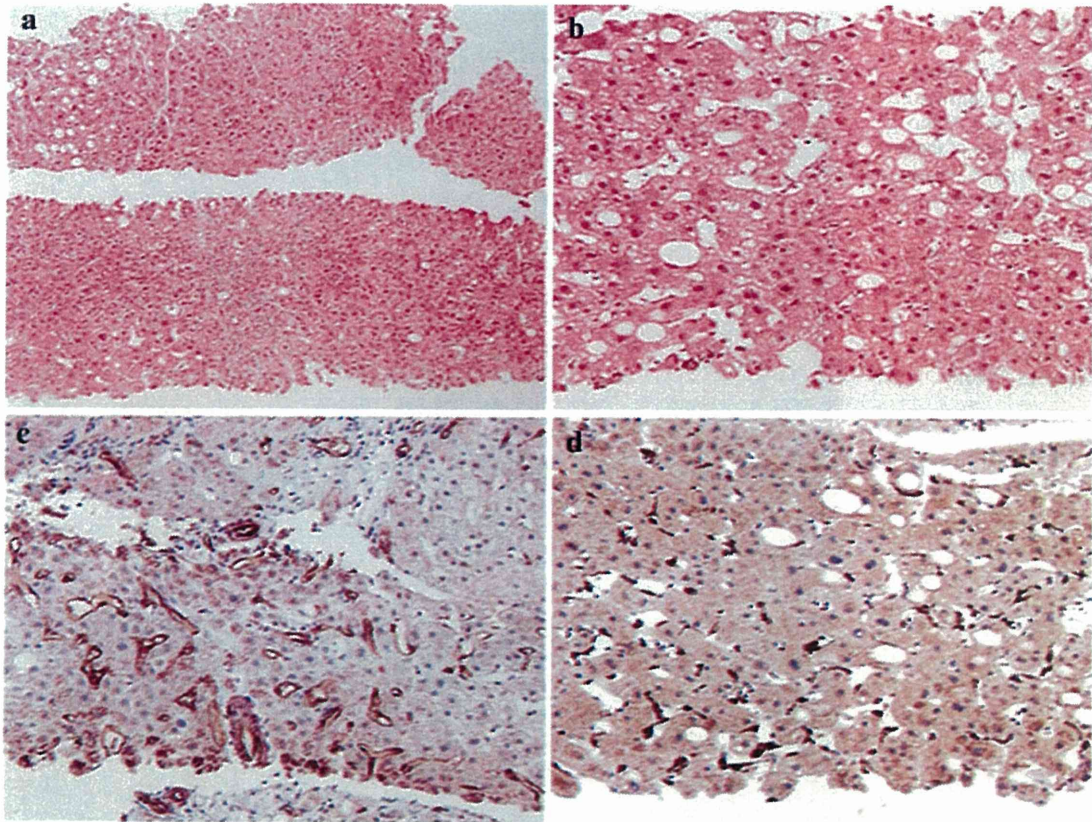


Fig. 3 Pathological findings of a liver biopsy in case 1. In comparison to the background liver tissue, the nodules showed a slightly increased cell density, scar-like fibrosis, and sinusoidal dilatation. Part of the nodule showed steatosis (a, b). The double

immunostain for both CD34 and alpha-smooth muscle actin showed a diffuse staining pattern in the sinusoidal endothelial cells, suggesting sinusoidal capillarization and unpaired arteries (c). Several CD68-positive Kupffer cells were detected in this nodule (d)

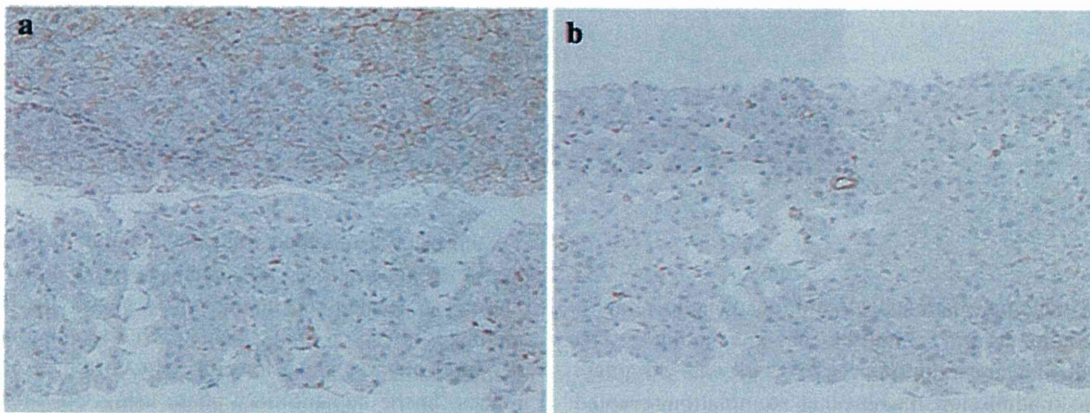


Fig. 4 The expression of OATP1B3 was observed predominantly in the cell membrane in the background liver in case 1 (a). However, part of this nodular lesion did not show any expression of OATP1B3 (b)

several CD68-positive Kupffer cells were observed in this nodule. These findings suggested a FNH-like nodule, arising from alcoholic cirrhosis, rather than a well-differentiated HCC.

Moreover, no immunostaining of OATP1B3 was observed in this nodule. These findings were consistent with the low intensity observed in the hepatobiliary phase of MRI (Fig. 7).

Fig. 5 Magnetic resonance imaging findings in case 2. The nodular lesion in liver S5 showed a high intensity at the precontrast T1 weighted image (a), was slightly enhanced at the early contrast phase of (b), was washed out at the portal phase (c), and had a defect in the hepatobiliary phase (d) (3D-GRE FS-T1W1, TR 4.30, TE 1.61, FA10)

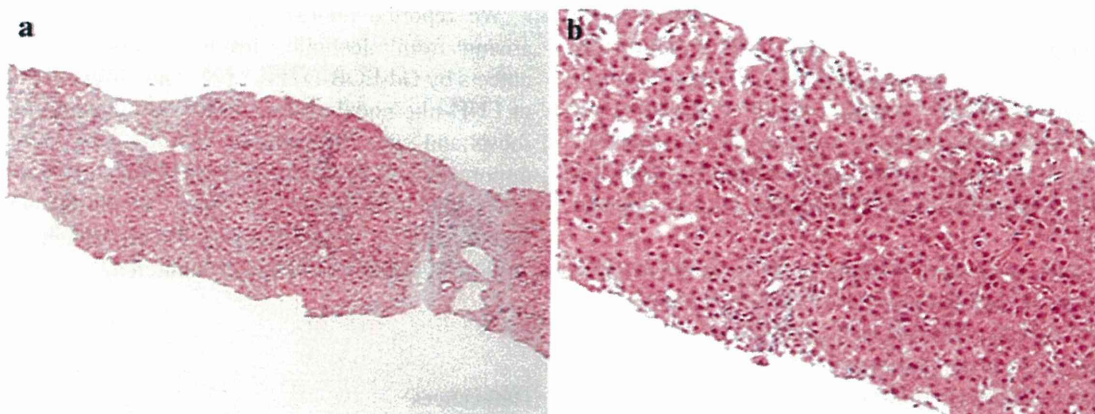
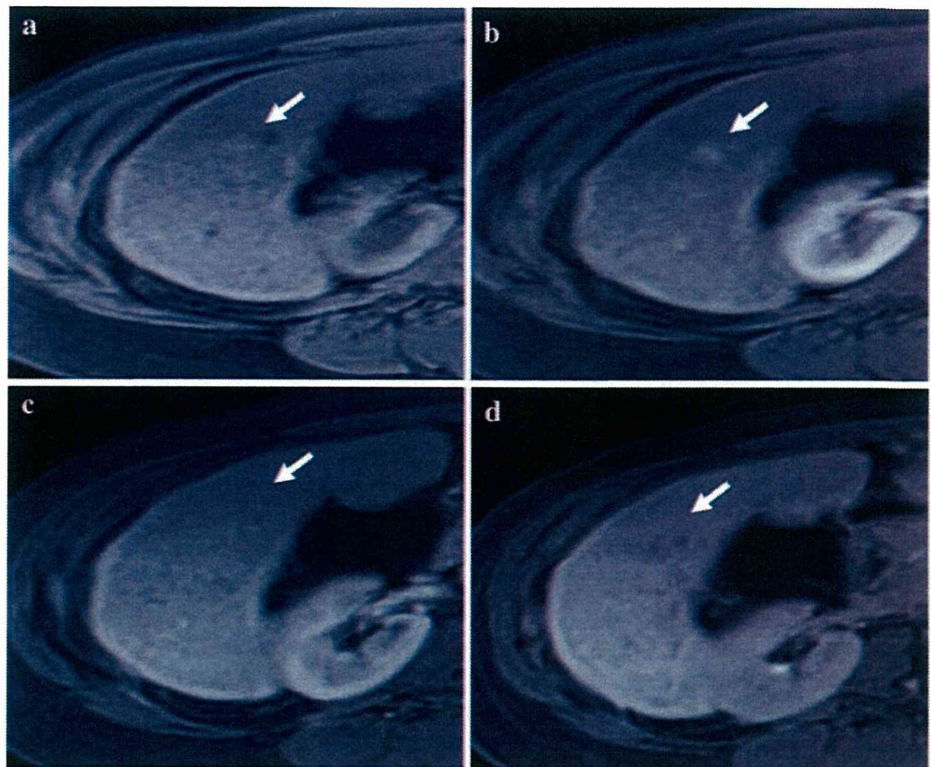


Fig. 6 Pathological findings of a liver biopsy in case 2. The background liver showed cirrhosis (a). The nodule showed a slightly increased cell density and sinusoidal dilatation in comparison to the background liver tissue (b)

Discussion

Although the International Working Party classified nodular hepatocellular lesions into several main classes, including regenerative lesions and dysplastic or neoplastic lesions [5], the most important clinical concern is the ability to distinguish between a regenerative lesion and a tumor lesion.

In the two current cases, HCC was first suspected from the imaging studies, which revealed a hypervascular tumor,

although hyperplastic nodules arising from alcoholic liver cirrhosis were considered in the differential diagnoses.

Regarding the tumor markers, PIVKA II was elevated in case 1 and AFP was elevated in case 2. AFP is a well-known tumor marker of HCC, and its diagnostic efficacy has been confirmed; however, its serum levels are also observed to increase in liver diseases such as chronic hepatitis and cirrhosis. Conversely, the serum PIVKA II level is significantly elevated in alcoholic liver disease compared to viral liver disease, although the mechanism of

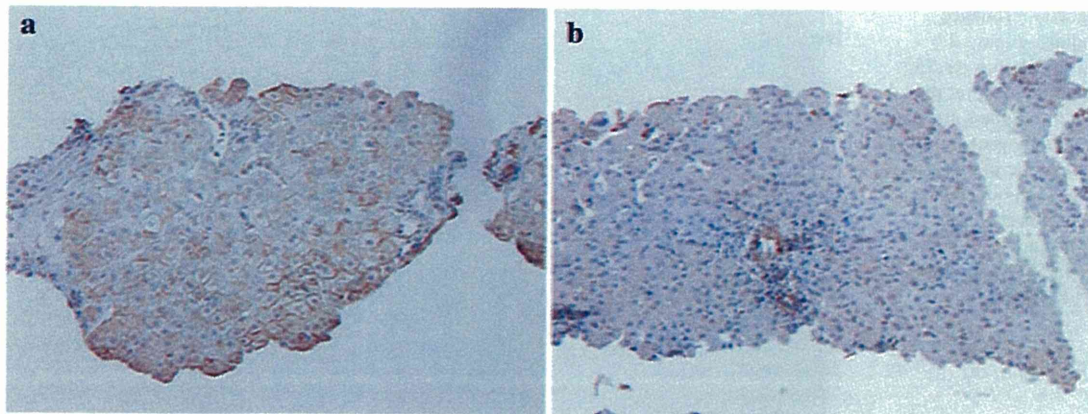


Fig. 7 The cell membrane of the background liver showed diffuse OATP1B3 in case 2 (a). This nodular lesion did not show any OATP1B3 expression (b)

the elevation of PIVKA II has not been clarified [6]. Ethanol intake and the following vitamin K deficiency may act, in part, due to an increased serum PIVKA II level in alcoholic liver disease. Taken together, tumor markers are not sufficient to differentiate between well-differentiated HCCs and FNH-like nodules arising in patients with alcoholic liver cirrhosis.

In case 1, the dynamic CT showed multiple nodular lesions with a delayed washout. In case 2, the dynamic CT showed multiple nodular lesions with arterial enhancement and a delayed washout. These findings were highly suggestive of a well-differentiated HCC. In most previous reports, these FNH-like nodules arising in patients with alcoholic liver cirrhosis lesions showed that the dynamic CT imaging resembles HCC, and furthermore, these two lesions are very difficult to differentiate from one another [1, 7–10].

Moreover, in case 1 the nodule in S2 showed encapsulation. While encapsulation is one of the typical findings in HCC, all FNH-like nodules in alcoholic liver cirrhosis are also reported to be encapsulated [1].

Recently, it has been reported that Gd-EOB-DTPA MRI has been helpful for the detection of small HCCs. In the present cases, most of the nodules showed defects in the hepatobiliary phase, suggesting HCC. Therefore, it is difficult to differentiate these hyperplastic nodules from well-differentiated HCCs using Gd-EOB-DTPA MRI alone. The difference in contrast following the administration of Gd-EOB-DTPA depends on the expression levels of the uptake transporter OATP1B3 [11] and excursion transporter (MRP2) [12]. The nodule in S2 showed partly positive expression of OATP1B3 in case 1. This finding was consistent with the MRI that showed slightly low intensity in the hepatobiliary phase. The S2 nodule in case 2 showed no OATP1B3 expression. These findings suggest that low

intensity in the hepatobiliary phase depends on the lack of OATP1B3 expression in FNH-like hyperplastic nodules.

Moreover, some reports have shown the Gd-EOB-DTPA uptake to be associated with pericellular fibrosis in nonalcoholic steatohepatitis [13, 14]. Pericellular fibrosis is the one of characteristic features of alcoholic liver disease. Therefore, the poor uptake of Gd-EOB-DTPA in our cases may depend on the degree of pericellular fibrosis in the nodule.

We reported on two patients with FNH-like nodules arising from alcoholic cirrhotic livers, which revealed defects by Gd-EOB-DTPA MRI. The differential diagnosis of FNH-like nodules in patients with alcohol-induced cirrhosis and well-differentiated HCCs is very difficult with tumor marker and imaging studies alone, such as dynamic CT and Gd-EOB-DTPA MRI. Therefore, histological confirmation is required when nodules arising in patients with alcoholic cirrhosis are encountered.

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